Mol. Cells *27*, 467-473, April 30, 2009
DOI/10.1007/s10059-009-0062-7

Molecules and Cells

Identification and Characterization of Alternative Promoters of the Rice MAP Kinase Gene *OsBWMK1*

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Our previous study suggested that *OsBWMK1*, a gene which encodes a member of the rice MAP kinase family, generates transcript variants which show distinct expression patterns in response to environmental stresses. The transcript variants are generated by alternative splicing and by use of alternative promoters. To test whether the two alternative promoters, *pOsBWMK1L* (promoter for the *OsBWMK1L* splice variant) and *pOsBWMK1S* (promoter for the *OsBWMK1S* splice variant), are biologically functional, we analyzed transgenic plants expressing *GUS* fusion constructs for each promoter. Both *pOsBWMK1L* and *pOsBWMK1S* are biologically active, although the activity of *pOsBWMK1S* is lower than that of *pOsBWMK1L*. Histochemical analysis revealed that *pOsBWMK1L* is constitutively active in most tissues at various developmental stages in rice and *Arabidopsis*, whereas *pOsBWMK1S* activity is spatially and temporally restricted. Furthermore, the expression of *pOsBWMK1S::GUS* was upregulated in response to hydrogen peroxide, a plant defense signaling molecule, in both plant species. These results suggest that the differential expression of *OsBWMK1* splice variants is the result of alternative promoter usage and, moreover, that the mechanisms controlling *OsBWMK1* gene expression are conserved in both monocot and dicot plants.

INTRODUCTION

Mitogen-activated protein kinases (MAPKs) play essential role in plant responses to biotic and abiotic stresses. The rice MAPK gene *OsBWMK1* is induced by plant defense signaling molecules as well as a fungal elicitor (Cheong et al., 2003). Recently, we reported that *OsBWMK1* transcript variants are generated by alternative splicing as well as the use of different transcriptional initiation sites (Koo et al., 2007). To date, many novel rice MAPKs have been isolated and their biological roles characterized (Agrawal et al., 2003; Fu et al., 2002; Huang et al., 2002; Song et al., 2002). However, transcriptional regulation of these genes is poorly understood.

The use of alternative first exons is one type of alternative splicing that most eukaryotes use to generate several transcripts from a single gene (Kazan, 2003; Kornblihtt, 2005; Lareau et al., 2004). Alternative first exons can be produced by alternative promoter usage. Frequently, alternative first exon transcripts arising by alternative promoter usage differ only in their 5′-untranslated region (UTR), share the same open reading frame, and produce identical proteins (Bonham et al., 2000; Kimura et al., 2006). For example, mouse *Bcl-X* isoforms are generated by alternative promoter usage (Viegas et al., 2004). The use of five promoters (P1-P5) gives rise to at least five mRNAs with different 5′-UTRs, all of which share the same translation initiation site. However, some alternative first exon transcripts contain different transcript start sites, which result in protein isoforms with different N-termini. These protein isoforms are localized to different subcellular compartments and, consequently, do function differentially (Liang et al., 2006; Wang et al., 2002; Yan et al., 2004).

To date, only a few studies have looked at alternative transcripts in plants. *SYN1* in *Arabidopsis* produces two transcripts with different 5′ ends: one transcript begins within the first intron of the other (Bai et al., 1999). In addition, the first intron of a rice MADS-box gene, *OsMADS1*, mediates preferential expression in flowers, whereas the 5′-upstream promoter region lacking first intron caused expression in both vegetative and reproductive tissues (Jeon et al., 2008). Recently, a large scale study of alternative first exons in rice and *Arabidopsis* discovered a number of potential alternative first exon-containing-clusters, which exhibit tissue- and/or development-specific transcription (Chen et al., 2007; Kitagawa et al., 2005). However, little is known about coupling mechanisms of alternative first exons and alternative promoter usage in plants.

Here, we report the isolation and characterization of two alternative promoters of the *OsBWMK1* gene. The results of the analysis of rice and *Arabidopsis* transgenic plants expressing

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Received January 19, 2009; revised February 24, 2009; accepted February 26, 2009; published online April 13, 2009

Keywords: alternative promoter, gene expression regulation, MAP kinase, rice, splice variant

GUS fusion constructs driven by the alternative promoters revealed that these two alternative promoters are biologically functional and are responsible for the distinct expression patterns of the splice variants. Thus, the expression of *OsBWMK1* may be regulated by alternative splicing coupled with the use of alternative promoters.

MATERIALS AND METHODS

Plasmid construction

Two putative promoter regions of *OsBWMK1*, a 987 bp fragment (*pOsBWMK1L*, from -987 to -1) and a 1251 bp fragment (*pOsBWMK1S*, from -128 to +1123), were amplified by PCR. Restriction sites, *Sma*I/*Nco*I in the *pOsBWMK1L* fragment and *Bam*HI/*Nco*I in the *pOsBWMK1S* fragment, were incorporated into primers to facilitate cloning. The amplified fragments were cloned into the pBluescript SK vector and confirmed by sequencing. The 987 bp and 1251 bp fragments were cloned in frame to the GUS report gene at the *Sma*I/*Nco*I or *Bam*HI/*Nco*I restriction sites, respectively, of the pCAMBIA 1301 vector. All clones were confirmed by sequencing. The constructs were introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation.

Transformation of rice and *Arabidopsis*

Rice calli (*Oryza sativa* L. cv. Dongjin) were transformed by *Agrobacterium*-mediated transformation as described by Lee et al. (1999). More than 10 independent primary transgenic lines were generated. T2 plant lines were used for analysis of reporter gene expression. *Arabidopsis* (ecotype Col-0) plants were transformed by the floral dip method (Clough et al., 1998) and transgenic seedlings were selected on MS medium containing 30 μg/ml hygromycin. More than 10 independent primary transgenic lines were generated. T3 plants were used for analysis of reporter gene expression.

Histochemical β-glucuronidase (GUS) staining

The GUS activity of transgenic T3 *Arabidopsis* plants and T2 rice plants was analyzed by histochemical staining using 5-bromo-4-chloro-3-indolyl glucuronic acid (X-gluc) as described by Jefferson et al. (1987). Briefly, whole plants at various stages were immersed in an X-gluc solution (1 mM X-gluc, 50 mM sodium phosphate (pH 7.0), 0.1% Triton X-100), and after applying vacuum for 5 min, were incubated at 37°C until satisfactory staining was observed. For better visualization of stained tissue, samples were rinsed, at room temperature, with an ethanol series for at least 1 h to remove chlorophyll, and then mounted for microscopy.

Quantitative GUS assay

A fluorometric GUS assay was performed using 4-methylumbelliferyl β-D-glucuronide (MUG) as a substrate, as described by Jefferson et al. (1987). Two-week-old transgenic *Arabidopsis* plants and 4-week-old transgenic rice plants were used for the GUS assay. Crude protein extracts were prepared by grinding samples in extraction buffer (50 mM sodium phosphate (pH 7.0), 1 mM EDTA, 10 mM β-mercaptoethanol) containing 0.1% Triton X-100, followed by centrifugation at 13,000 × *g* for 10 min. The GUS activity in supernatants was measured in extraction buffer containing 1 mM MUG. Reactions were stopped by the addition of 0.2 M Na_2CO_3 and fluorescence was then measured.

H_2O_2 treatment

Detached leaves from transgenic rice plants expressing *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* were incubated in 10 mM $MgCl_2$ and 0.01% silwet L-77 with 2 mM H_2O_2 as described by Agrawal et al. (2000). After incubation for 3 h, samples were frozen in liquid nitrogen and stored at -70°C. Two-week-old transgenic *Arabidopsis* plants were infiltrated by dipping in 10 mM $MgCl_2$ and 0.01% silwet L-77 with 2 mM H_2O_2. Three independent plants were harvested at the indicated times (0, 2, 6 and 12 h), frozen in liquid nitrogen, and stored at -70°C until used.

Western blot analysis

Crude protein extracts were isolated from transgenic rice plants as previously described (Cheong et al., 2003) and separated by 10% SDS-PAGE. The pCAMBIA 1301 vector contains a *GUS* reporter gene fused to a 6x His tag and a commercial anti-His-HRP antibody (Abcam) was used to detect GUS expression. Western blot analysis was performed as described by Koo et al. (2007).

Northern blot analysis

Total RNA was isolated from transgenic *Arabidopsis* plants as described by Cheong et al. (2003), and 20 μg of total RNA was subjected to electrophoresis through a 1.5% (w/v) formaldehyde agarose gel and transferred onto a nylon membrane (Hybond-N^+, Amersham). The membrane was hybridized to a ^{32}P-labeled full-length *GUS* probe. Hybridization was carried out at 65°C for 18 h in Church's buffer (1% BSA, 1 mM EDTA, 0.5 M $NaHPO_4$ (pH 7.2), 7% SDS). The membrane was washed in 0.1× SSC, 0.1% SDS at 50°C and exposed to X-ray film (Kodak).

RESULTS

Identification of two alternative promoters of the *OsBWMK1* gene

We have previously shown that three transcript variants (named *OsBWMK1L, OsBWMK1M* and *OsBWMK1S*) are generated from the *OsBWMK1* gene by alternative splicing events. Expression of the *OsBWMK1M* and *OsBWMK1S* transcripts is inducible in response to plant defense signaling molecules, such as hydrogen peroxide (H_2O_2), salicylic acid (SA), and jasmonic acid (JA), while expression of *OsBWMK1L* is constitutive (Koo et al., 2007). Moreover, the differential expression patterns of the *OsBWMK1* transcript variants may be due to the utilization of two alternative promoters: one may be responsible for constitutive expression of *OsBWMK1L* and the other for inducible expression of *OsBWMK1M* and *OsBWMK1S*. As a result of alternative splicing, *OsBWMK1S* has an alternative translation start site at nucleotide position +1124, corresponding to exon 2 of the *OsBWMK1* gene. To test whether the region upstream of the alternative translation start site of *OsBWMK1S*, which includes part of the 5′ untranslated region (UTR), exon 1, and intron 1 of *OsBWMK1*, regulates gene expression, we cloned approximately 1 kb up-stream of the translation start sites of the *OsBWMK1L* and *OsBWMK1S* splice variants using genomic PCR. The PCR fragments were designated *pOsBWMK1L* (Promoter for *OsBWMK1L*; GenBank accession number, FJ628390) and *pOsBWMK1S* (Promoter for *OsBWMK1S*; GenBank accession number, FJ628391), respectively (Fig. 1). Our previous study suggested that *OsBWMK1M* and *OsBWMK1S* are generated by the same promoter and *OsBWMK1M* then undergoes further alternative splicing (Koo et al., 2007). The cloned promoter region for *OsBWMK1L* contains 987 bp of the 5′-upstream region and that for *OsBWMK1S* consists of 128 bp of the 5′-UTR, exon 1, and intron 1 of *OsBWMK1* (Fig. 1). To test whether the cloned *pOsBWMK1L* and *pOsBWMK1S* fragments

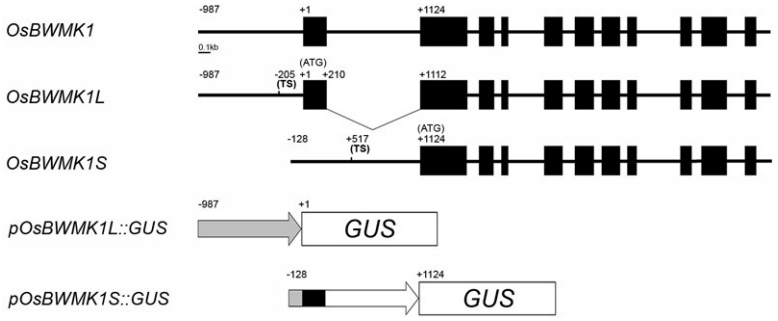

Fig. 1. Schematic representation of GUS fusion constructs carrying putative promoter regions of the *OsBWMK1* transcript variants. Genomic structure and two transcript variants of the *OsBWMK1* gene are shown. The numbering of the nucleotides is based on the first translation start site in *Os BWMK1* (numbered +1). The transcriptional start sites (TS) and translation start codons (ATG) of the *OsBWMK1L* and *OsBWMK1S* transcripts are marked. The scale bar is 0.1 kb. The 987 bp upstream region *pOsBWMK1L* and the 1251 bp upstream region *pOsBWMK1S*, which consists of 5′-UTR sequence (gray box), exon 1 (black box) and intron 1 (white box) were each fused to *GUS* gene constructs.

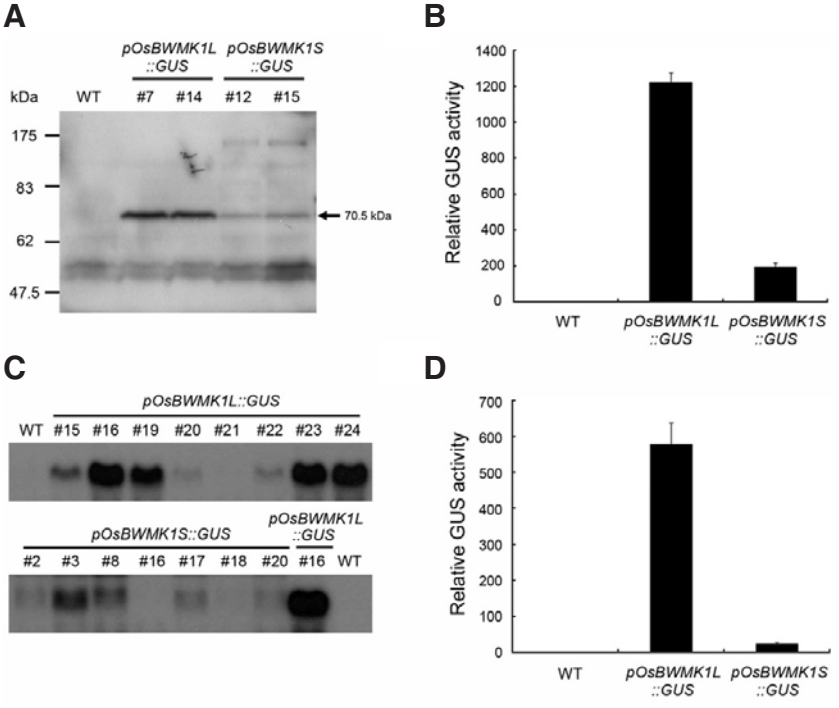

Fig. 2. GUS activity of the two putative promoter regions of *OsBWMK1* in transgenic rice and *Arabidopsis* plants. (A) Western blot analysis of *pOsBWMK1L::GUS* and *pOs BWMK1S::GUS* expression in T2 transgenic rice plants. (B) Fluorometric GUS enzyme assay. GUS enzyme activity was measured in leaves of 2-week-old T2 transgenic rice plants (line 7 for *pOsBWMK1L::GUS* and line 15 for *pOsBWMK1L::GUS*) expressing *pOsBWMK1L::GUS* or *pOs BWMK1S::GUS.* (C) Northern blot analysis of *pOsBWMK1L:: GUS* and *pOsBWMK1S::GUS* transgenic *Arabidopsis* plants. *GUS* expression in transgenic lines (T2) was detected by Northern blotting. (D) GUS enzyme activity was measured fluorometrically in 2-week-old transgenic *Arabidopsis* seedlings (T3).

can function as promoters, we fused these two fragments to the *GUS* reporter gene to generate *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* (Fig. 1).

Generation of transgenic rice and *Arabidopsis* plants expressing the *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* constructs

Two *GUS* fusion constructs, *pOsBWMK1L::GUS* and *pOs BWMK1S::GUS*, were introduced into rice embryogenic calli by *Agrobacterium*-mediated transformation. Hygromycin-resistant transgenic rice lines were further selected by Northern blot analysis using *GUS* as a probe to isolate transgenic rice lines showing the highest expression of *GUS*. Two independent T2 lines were selected for each construct (data not shown). The expression patterns of *pOsBWMK1L::GUS* and *pOsBWMK1S:: GUS* were further analyzed by Western blot analysis using anti-His-HRP to confirm the stable accumulation of GUS protein, which is fused in-frame to a 6x His tag, and driven by either *pOsBWMK1L* or *pOsBWMK1S*. As shown in Fig. 2A, the antibody strongly cross-reacted with a band corresponding to the expected molecular weight (70.5 kDa) of the fusion protein in *pOsBWMK1L::GUS* transgenic rice plants. Although the signal was weak, we were able to see the accumulation of GUS in *pOsBWMK1S::GUS* transgenic rice plants at the same position, indicating that *pOsBWMK1L* is more active than *pOsBWMK1S*. In *pOsBWMK1S::GUS* transgenic rice plants, the antibody cross-reacted with higher molecular weight proteins migrating between 83 and 175 kDa. These bands may be due to enrichment of nonspecific proteins during the preparation of crude protein from *pOsBWMK1S::GUS* transgenic rice plants or to the formation of GUS multimers. The Western blotting results indicate that both *pOsBWMK1L* and *pOsBWMK1S* are sufficient to drive expression of the downstream *GUS* gene (Fig. 2A). Quantitative assessment of the activities of the two promoter fusions using a fluorometric GUS assay revealed that *pOsBWMK1L* was approximately 6-fold more active than *pOs BWMK1S* in transgenic rice plants (Fig. 2B) and these differential promoter activities of *pOsBWMK1L* and *pOsBWMK1S* are consistent with the different transcript levels of *OsBWMK1L* and *OsBWMK1S* splice variants in rice plants determined by RT-PCR analysis in our previous report (Koo et al., 2007).

We introduced the *pOsBWMK1L::GUS* and *pOsBWMK1S:: GUS* constructs into *Arabidopsis* plants to examine whether the rice promoters are functional in a heterologous dicotyledonous

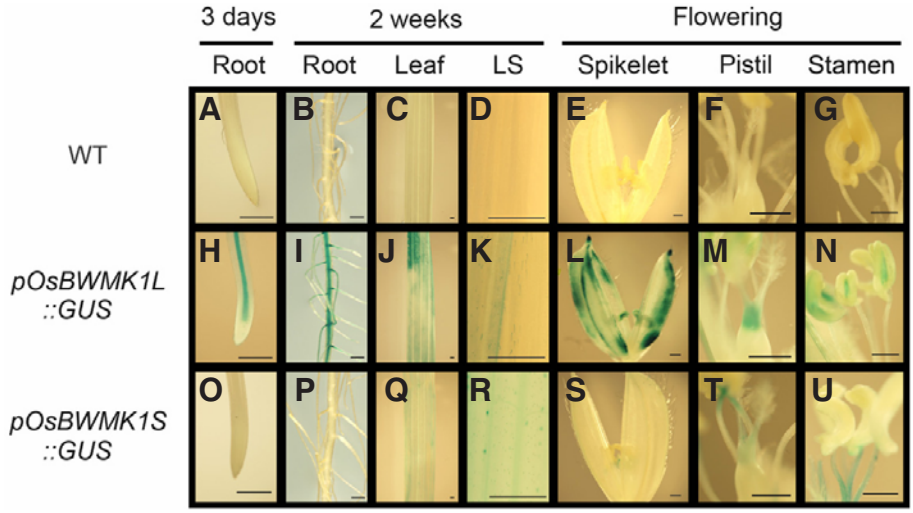

Fig. 3. Histochemical analysis of *pOs BWMK1L::GUS* and *pOsBWMK1S:: GUS* protein levels in transgenic rice plants. The GUS activity of wild-type (WT, A-G), *pOsBWMK1L::GUS* (H-N), and *pOsBWMK1S::GUS* (O-U) in various tissues of the transgenic rice plants at different developmental stages was analyzed. (A, H, and O), the apical region of the primary root 3 days post-germination. (B, I, and P), roots at 2 weeks postgermination. (C, J, and Q), leaves at 2 weeks post-germination. (D, K, and R), trichomes in the leaf sheathes (LS) at 2 weeks post-germination. (E, L, and S), mature spikelets before anthesis at flowering. (F, M, and T), stigmas and styles of the pistils at flowering. (G, N, and U), anthers and filaments of the stamens at flowering. The scale bars indicate 500 μm.

system. Northern blot analysis indicated that the promoter activity of *pOsBWMK1L* is stronger than that of *pOsBWMK1S* in *Arabidopis* (Fig. 2C), similar to the trends observed in rice. A quantitative GUS assay conducted with T3 lines of *pOs BWMK1L::GUS* and *pOsBWMK1S::GUS* transgenic *Arabidopsis* plants exhibiting the strongest *GUS* expression revealed that *pOsBWMK1L* is approximately 25-fold more active than *pOsBWMK1S* in *Arabidopsis* (Fig. 2D). Although the relative expression levels of *pOsBWMK1L::GUS* and *pOsBWMK1S:: GUS* differ in transgenic rice and *Arabidopsis* plants, the relative trends in promoter activity were well conserved in mono- and dicotyledonous plant species. Taken together, these results suggest not only that both *pOsBWMK1L* and *pOs BWMK1S* are functionally active promoters, but also that the regulatory mechanism for the activities of *pOsBWMK1L* and *pOsBWMK1S* are well conserved between mono- and dicotyledonous species.

Spatial and temporal expression patterns of *pOsBWMK1L:: GUS* and *pOsBWMK1S::GUS* in transgenic rice and *Arabidopsis* plants

To determine the spatial and temporal expression patterns of *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS*, we examined GUS staining in various tissues at different developmental stages in 3-day-old, 2-week-old, and flowering transgenic rice plants (Fig. 3). In *pOsBWMK1L::GUS* plants GUS activity was detected at high levels in most tissues regardless of developmental stage (Figs. 3H-3N). In contrast, we observed highly localized and weak GUS staining in particular developmental stages in *pOsBWMK1S::GUS* plants (Figs. 3O-3U). Wild-type rice plants did not exhibit any background GUS staining (Figs. 3A-3G). In the vegetative stages, *pOsBWMK1S::GUS* activity was only weakly detected in the leaves and trichomes of 2-week-old transgenic plants (Figs. 3Q and 3R), while strong *pOsBWMK1L::GUS* activity was detected in roots, leaves, and trichomes (Figs. 3H-3K). In the reproductive organs, *pOs BWMK1L::GUS* expression was broadly distributed, being observed in the lemma, palea, veins of the spikelets, and the lower parts of styles and anthers (Figs. 3L-3N). In contrast, *pOsBWMK1S::GUS* expression was only detected in the male and female reproductive organs, especially in the upper parts of the styles and the filaments of the stamens (Figs. 3S-3U). These results indicate that the two alternative promoters of the *OsBWMK1* gene control the distinctive expression of *Os BWMK1L* and *OsBWMK1S*, not only determining the transcript levels, but also the spatial and temporal expression patterns.

The expression patterns of *pOsBWMK1L::GUS* and *pOs BWMK1S::GUS* in transgenic *Arabidopsis* plants were similar to those observed in transgenic rice plants (Fig. 4). In the vegetative stage, strong GUS staining was observed in most organs of *pOsBWMK1L::GUS Arabidopsis* plants (Figs. 4E-4G). However, GUS staining in the vegetative tissues of *pOsBWMK1S:: GUS Arabidopsis* plants was only detected in the trichomes (Figs. 4I-4L). Wild-type *Arabidopsis* plants did not exhibit GUS staining (Figs. 4A-4D). In the reproductive organs, *pOsBWMK1L::GUS* expression was evident in stigmas, filaments, and the vasculature of the sepals and pedicels (Fig. 4H), but *pOsBWMK1S:: GUS* expression was only detected in anthers (Fig. 4L). These results suggest that the regulatory mechanisms controlling the expression patterns of *pOsBWMK1L::GUS* and *pOsBWMK1S:: GUS* is highly conserved between *Arabidopsis* and rice. However, the control of the spatial expression of *pOsBWMK1L:: GUS* and *pOsBWMK1S::GUS* in floral organs may be distinct between mono- and dicotyledonous species.

Differential regulation of *pOsBWMK1L* and *pOsBWMK1S* activity in response to H_2O_2

Our previous report indicated that gene expression of the *OsBWMK1S* splice variant is highly inducible in response to fungal elicitor, plant defense signaling molecules (H_2O_2, SA, JA) and salt stress, while expression of the *OsBWMK1L* splice variant is constitutive (Koo et al., 2007). To examine whether the two alternative promoters of *OsBWMK1* react differently to environmental stresses, we treated transgenic rice and *Arabidopsis* plants expressing *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* with H_2O_2. As shown in Fig. 5A, GUS activity driven by *pOs BWMK1S* in rice transgenic plants was significantly upregulated (about 1.3 fold) in response to H_2O_2 (Student's *t*-test, $P < 0.05$). The activity of *pOsBWMK1L* was not affected by H_2O_2 treatment. We also consistently observed an upregulation in *pOsBWMK1S::GUS* expression in response to H_2O_2 in transgenic *Arabidopsis* plants (Fig. 5B). Expression of *pOs BWMK1S::GUS* was increased by approximately 1.9 fold in response to H_2O_2, while *pOsBWMK1L::GUS* expression was unaltered. These results are consistent with our previous study, which revealed that *OsBWMK1S* was upregulated by various

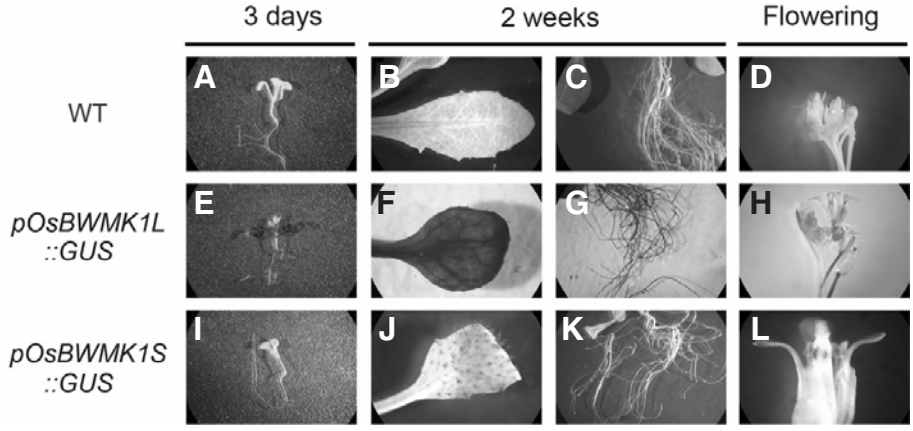

Fig. 4. Histochemical analysis of GUS activity in *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* trangenic *Arabidopsis* plants. The GUS activity of wild-type (WT, A-D), *pOs BWMK1L::GUS* (E-H) and *pOs BWMK1S::GUS* (I-L) in various tissues of the transgenic *Arabidopsis* plants at different developmental stages was analyzed. (A, E, and I), whole plants at 3 days post-germina-tion. (B, F, and J), leaves at 2 weeks post-germination. (C, G, and K), roots at 2 weeks post-germination. (D, H, and L), mature flowers.

stressors, but *OsBWMK1L* was abundantly and constitutively expressed (Koo et al., 2007).

DISCUSSION

We showed previously that alternative splicing of *OsBWMK1* generates three different transcripts that produce proteins with different subcelluar localizations (Koo et al., 2007). Moreover, these transcript variants use different translation start sites and the expression patterns of the transcripts are distinct, suggesting that the expression of alternative splice variants of *Os BWMK1* gene can be differentially regulated by alternative promoters. To test this hypothesis, we characterized the 5′-upstream regions of the *OsBWMK1L* and *OsBWMK1S* transcripts using transgenic rice and *Arabidopsis* plants carrying GUS fusions to the putative promoter regions. The expression analysis showed that two upstream regions (*pOsBWMK1L* and *pOsBWMK1S*) of the gene exhibit biologically active promoter functions in both transgenic rice and *Arabidposis* plants, suggesting that *OsBWMK1* transcript variants are generated by using different promoters as well as by alternative splicing events. Many eukaryotic genes contain multiple promoters. Each promoter determines a specific transcription start site and first exon, and, consequently, generates a different transcript. Generation of alternative transcripts by the use of multiple promoters is an additional way to create regulatory diversity and provides a mechanism to coordinate the synthesis of functionally related proteins that must act together to mediate a certain biological response (Morello et al., 2002; Parsley et al., 2006; Qi et al., 2007).

The distinct expression patterns of the *OsBWMK1L* and *OsBWMK1S* transcripts, as well as the *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* constructs, prompted us to analyze the nucleotide sequences of both promoters. Sequence analysis of the *pOsBWMK1L* and *pOsBWMK1S* promoters using the PlantCARE software (Lescot et al., 2002) revealed that *pOs BWMK1L* contains a TATA rich region, which is a known transcriptional enhancer, and is consistent with the constitutively active expression patterns of both the *OsBWMK1L* transcript and *pOsBWMK1L::GUS* (Fig. 6). In addition, the *pOsBWMK1S* promoter contains several interesting putative *cis*-acting elements which are responsive to biotic stress, including a W-box and a CGTCA-element (a methyl jasmonate (MeJA) response element). The W-box motif containing the core nucleotide sequence TGAC has been identified as a binding site of the plant specific WRKY transcription factors (Eulgem et al., 1999). The expression of *WRKY* is induced by several plant defense signaling molecules such as MeJA, SA, and ethylene, as well as wounding and pathogen infection, suggesting that WRKY might participate in plant defense signaling against pathogens (Asai et al., 2002; Eulgem et al., 2007; Johnson et al., 2002; Liu et al., 2007). We demonstrated previously that the expression of the *OsBWMK1S* transcript is induced by plant stressors such as fungal elicitor, H_2O_2, SA, JA, and NaCl in rice (Koo et al., 2007). In agreement with these previous results, only the activity of *pOsBWMK1S* was increased by H_2O_2 treatment, in both rice and *Arabidopsis* plants. Thus, an interaction between a W-box

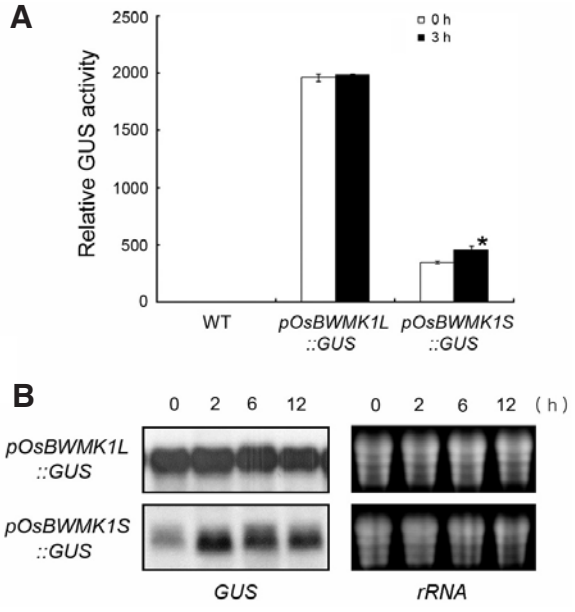

Fig. 5. Effect of H_2O_2 on expression of *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* in transgenic rice and *Arabidopsis* plants. (A) The GUS activity of *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* transgenic rice plants in response to H_2O_2 determined in three independent lines by fluorometric analysis. The asterisk indicates a significant difference (Student's *t*-test, $P < 0.05$) compared to untreated controls (0 h). (B) Northern blot analysis of *GUS* expression in *pOsBWMK1L::GUS* and *pOsBWMK1S::GUS* transgenic *Arabidopsis* plants in response to H_2O_2. Total RNA was isolated from whole plants treated with H_2O_2 for the indicated times (0, 2, 6, 12 h). *GUS* expression was analyzed by Northern blotting with a *GUS*-specific probe.

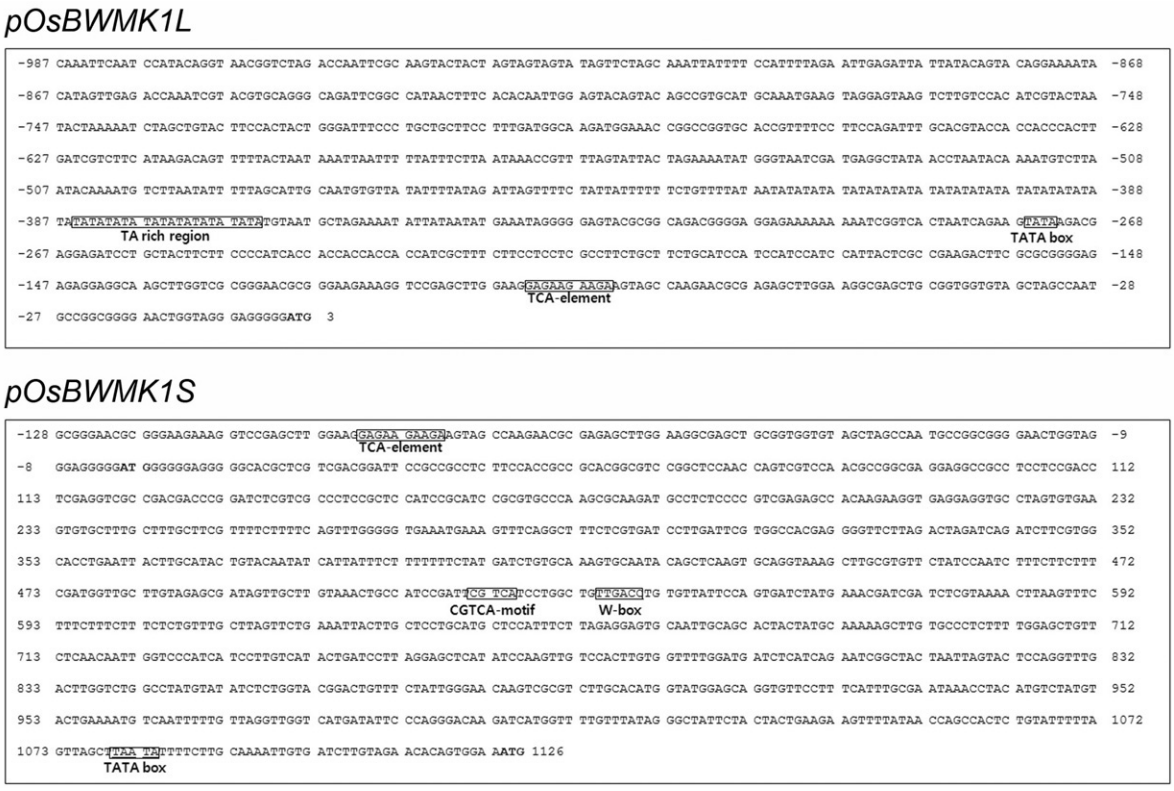

Fig. 6. The nucleotide sequences of the two alternative promoters of the *OsBWMK1* gene showing putative *cis*-acting elements. Potential *cis*-acting elements predicted by PlantCARE are boxed in both the *pOsBWMK1L* and *pOsBWMK1S* promoters. A TATA box, TATA rich region (enhancer element), TCA-element (SA responsive element), CGTCA-motif (MeJA responsive element), and W-Box (WRKY binding element) are indicated.

motif in *pOsBWMK1S* and a WRKY transcription factor may play a role in stress-induced *OsBWMK1S* expression in rice. In both transgenic rice and *Arabidopsis*, *pOsBWMK1S::GUS* is strongly expressed in trichomes, which frequently function as the first line of defense against pathogen attack, either by inducing spatial hindrance or physical entrapment, or by secreting toxic or behavior-modifying chemicals (Li et al., 2004; Wagner et al., 2004). Moreover, such trichome-based defenses play an important role in the development of sustainable pest control strategies (Lewis et al., 1997).

There are very few examples of monocot promoters that are also functional in dicots (Iwamoto et al., 2004; Liu et al., 2003; Tittarelli et al., 2007). Our results demonstrate that the expression patterns of two alternative promoters of *OsBWMK1* are highly conserved in both rice and *Arabidopsis*, suggesting that the regulatory mechanism of MAP kinase transcription may be conserved between monocot and dicot plant species.

In this report, we have shown that two alternative promoters of *OsBWMK1* play different roles in controlling the level of transcription and in the disparate spatial and temporal expression patterns of *OsBWMK1* expression in rice and *Arabidopsis*, which is consistent with the presence of a distinct set of *cis*-acting elements in these two promoters. The results suggest that expression of *OsBWMK1* transcripts could be regulated by different sets of transcriptional apparati in response to different environmental conditions. Identifying the regulatory component(s) involved in the inducible expression of the *OsBWMK1S* splice variant will provide new insights of MAPK-mediated defense signaling cascades in plants.

ACKNOWLEDGMENTS

This work was supported by grants from the Environmental Biotechnology National Core Research Center (R15-2003-012-01001-0) funded by the Ministry of Education, Science and Technology of Korea, the Basic Research Program (R01-2006-000-10035-0) of Korea Science and Engineering Foundation, and the Biogreen 21 Project (2007 0301034030) of the Rural Development Administration. SCK and HJC were supported by scholarships from the Brain Korea 21 Program of the Ministry of Education, Science and Technology of Korea.

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